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Antibodies to JC and BK viruses among persons with non-Hodgkin lymphoma

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Two related polyomaviruses, JC virus (JCV) and BK virus (BKV), commonly cause lifelong infections in humans, with periodic reactivation manifesting as viral shedding in urine. Because JCV can infect lymphocytes and cause chromosomal damage, it is a plausible candidate to cause non-Hodgkin lymphoma (NHL). To test this hypothesis, we measured IgG antibodies to JCV and BKV capsids using a virus-like particle enzyme immunoassay in 3 separate groups of subjects. First, in a U.S. population-based case-control study of NHL (724 cases, 622 controls), we found lower JCV antibody levels in cases than controls (median optical density = 0.12 *vs.* 0.21, $p < 0.0001$); likewise, JCV seroprevalence was lower in cases (49% *vs.* 59%, adjusted odds ratio [OR] = 0.70, 95% confidence interval [CI] = 0.56–0.87). In contrast, BKV antibody levels did not differ between groups. Second, we found that JCV and BKV antibody levels changed little over time among 24 NHL patients receiving chemotherapy. Third, we evaluated 126 homosexual men, of whom 46 were shedding JCV and 14 were shedding BKV in urine. Antibody levels were much higher in shedders than non-shedders (JCV: median optical density = 0.67 *vs.* 0.07, $p < 0.0001$; BKV: 0.87 *vs.* 0.40, $p = 0.003$), indicating that these antibodies are a marker for viral replication. Because no deficit of BKV antibody was seen in NHL cases, and because antibody levels did not change materially with chemotherapy, we suggest that the lower levels of JCV antibody observed in NHL patients may not be due entirely to a disease or treatment effect. Additional research is needed to determine whether JCV replication is decreased in individuals with NHL and whether these findings are consistent with an etiologic role for JCV in NHL.

Key words: JC virus; BK virus; non-Hodgkin lymphoma; epidemiology; case-control study

Two polyomaviruses, JC virus (JCV) and BK virus (BKV), commonly infect humans.[1–3] After initial infection, both viruses establish lifelong latency in the kidney, with periodic episodes of reactivation manifesting as shedding of virus in urine.[4] Mechanisms by which chronic polyomavirus infections are controlled are incompletely understood, although the cellular immune system is likely important.[4] JCV and BKV infections are largely asymptomatic, but severe disease can develop with immunosuppression, *e.g.*, JCV can cause progressive multifocal leukoencephalopathy in persons with acquired immunodeficiency syndrome (AIDS), and BKV can cause interstitial nephritis in kidney transplant recipients.[4] BKV and JCV can both induce malignancies when injected into experimental animals,[5] prompting research interest regarding whether these viruses contribute to cancer in humans.

In some respects, JCV represents a plausible candidate as a cause of non-Hodgkin lymphoma (NHL) in humans. The etiology of NHL is largely unknown, although several viruses, such as Epstein Barr virus and human immunodeficiency virus (HIV), play a role in some subtypes.[6] JCV can infect B lymphocytes[7] and induces chromosomal damage in some experimental cell lines (*e.g.*, colon cancer cells).[8] JCV antibody levels are elevated in individuals who possess circulating ''rogue'' cells, *i.e.*, ''cultured lymphocytes exhibiting extreme chromosomal damage.''[9] Nonetheless, the possible association between JCV and NHL has not been studied extensively.

In our present study, we sought to determine whether patterns of antibody reactivity to JCV and BKV might indicate that either virus plays an etiologic role in NHL. Three separate groups of subjects were studied using virus-like particle enzyme immunoassays (VLP EIA) developed recently, which detect IgG antibodies to JCV and BKV capsids.[10,11] First, we evaluated NHL cases and controls from a large U.S. population-based case-control study, to determine whether antibody patterns differed in persons with NHL. Second, we measured serial antibody levels among a separate group of NHL patients undergoing chemotherapy to determine whether antibody levels change with treatment. Finally, to put these results into context, we studied a group of homosexual men, some of whom were shedding JCV or BKV in their urine, and demonstrate that high-level IgG antibody to the capsid of these viruses is a marker for the presence of active viral replication.

Material and methods

Study subjects

The National Cancer Institute-Surveillance, Epidemiology and End Results (NCI-SEER) case-control study was described previously.[12,13] During 1998–2000, HIV-negative subjects were enrolled from 4 U.S. areas covered by the SEER Program: Iowa state, and the Detroit, Los Angeles, and Seattle metropolitan areas. Cases (n = 1,321) were individuals identified by the registries as having incident NHL (ages 20–74 years). Controls (n = 1,057) were recruited from the general population in the 4 areas, frequency matched to cases by age, gender and ethnic origin, and identified using random digit dialing (ages 20–64 years) or Medicare eligibility files (ages 65–74 years). The present investigation includes 1,346 subjects (724 cases and 622 controls) for whom serum was available.[13] Evaluated subjects resembled those who were excluded due to lack of serum (data not shown).

To examine the effect of chemotherapy on viral antibody levels, we studied 24 additional HIV-uninfected patients (17 male, 7 female; median age = 50 years) with newly diagnosed large B cell NHL. These individuals participated in a separate NCI-sponsored study of etoposide, prednisone, vincristine, cyclophosphamide and doxorubicin (EPOCH) chemotherapy at 3 U.S. sites in 1997–2000.[14] Investigators obtained blood specimens from each

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TABLE I – DEMOGRAPHIC CHARACTERISTICS OF SUBJECTS FROM THE NCI-SEER CASE-CONTROL STUDY OF NON-HODGKIN LYMPHOMA[1]

Category	Cases (n = 724)	Controls (n = 622)	p-value[2]
Gender			0.82
Male	399 (55.1)	339 (54.5)	
Female	325 (44.9)	283 (45.5)	
Age at diagnosis or selection, years			0.04
20–34	38 (5.3)	39 (6.3)	
35–44	96 (13.3)	60 (9.7)	
45–54	155 (21.4)	115 (18.5)	
55–64	195 (26.9)	137 (22.0)	
65–74	240 (33.2)	271 (43.6)	
Mean age (SD)	56.7 (12.4)	58.1 (12.6)	
Ethnic origin			0.08
Caucasian	635 (87.7)	536 (86.2)	
African-American	40 (5.5)	52 (8.4)	
Other	49 (6.8)	34 (5.5)	
Center			0.71
Detroit	76 (10.5)	66 (10.6)	
Iowa	248 (34.3)	212 (34.1)	
Los Angeles	167 (23.1)	129 (20.7)	
Seattle	233 (32.2)	215 (34.6)	
Education, years			0.31
0–11	62 (8.6)	54 (8.7)	
12–15	463 (64.0)	374 (60.1)	
16 or more	199 (27.5)	194 (31.2)	

[1]Values for cases and controls are number (%), except where stated (n = 1,346).–[2]p-Values were derived using χ^2 test, except for age, where 2-sample t-test was used.

patient at 4 timepoints (1 pre-therapy, 2 during therapy, 1 post-therapy), but the number of subjects for whom serum was available varied by timepoint (mean = 2.8 samples/patient; n = 18, n = 17, n = 17 and n = 15 patients, respectively, for the 4 timepoints). In persons with paired serum specimens, the median time from the first to the second timepoint was 42 days (n = 13 subjects), the median time from the second to the third sample was 63 days (n = 13), and the median time from the third to the fourth sample was 105 days (n = 11).

Finally, we studied 126 subjects from a cohort of homosexual men recruited in Washington, D.C., and New York City at the start of the HIV epidemic in 1982.[15] Urine specimens from these men had been tested previously by polymerase chain reaction for the presence of JCV and BKV DNA.[16] In our present study, we measured JCV and BKV antibody levels in serum samples obtained simultaneously with these urine specimens. At the time of sampling (1986–96), the median age of the subjects was 37 years. Forty-nine (39%) were HIV-infected and had a wide spectrum of CD4 counts consistent with varying degrees of immune suppression (CD4 count 0–249 cells/mm^3, n = 11; CD4 count 250–499 cells/mm^3, n = 18; CD4 count 500+ cells/mm^3, n = 20).

Serological testing for JCV and BKV antibodies

JCV and BKV VLP were generated from VP1 proteins expressed in insect cells.[10] EIA plates (PolySorp, Nunc, Naperville, IL) were incubated overnight with VLP (30 ng/well). Serum specimens (1:400 dilution) were then tested in duplicate according to a standard EIA protocol.[11] After development of the EIA reaction with 2,2′-azino-di-(3-ethylbenzthiazoline-6-sulfonate) hydrogen peroxide solution, absorbance was measured at 405 nm.

We utilized the geometric mean of the duplicate absorbance measurements in our analyses and applied a cutoff of 0.12 optical density (OD) units to define JCV and BKV seropositivity; use of slightly different alternative cutoffs yielded similar results (data not shown). Because there is no standard test for JCV and BKV infection, the sensitivity and specificity of these assays is unknown. Nonetheless, the assays are identical in format to a VLP EIA concurrently developed for simian virus 40 (SV40), a related monkey polyomavirus.[10] The SV40 EIA and its cutoff of 0.12 OD units were validated using samples from experimentally infected primates and had 100% sensitivity and 100% specificity for detecting neutralizing antibody positive SV40 infection in primates.[11] The EIA for JCV and BKV each do not detect antibody against the other human polyomavirus, and both demonstrate little cross-reactivity against SV40.[10,13] Additionally, we used our data on homosexual men to choose a higher cutpoint for the JCV EIA (0.30 OD units, ''high-level JCV antibody'') that discriminated between JCV shedders and non-shedders.

Statistical methods

We compared demographic characteristics of cases and controls from the NCI-SEER study, using the χ^2 and t-tests. To characterize JCV and BKV antibody patterns in these subjects, we present box plots of the EIA absorbances, and we tested for differences between cases and controls with the Wilcoxon rank sum test. Using the EIA cutoff of 0.12 OD units, we classified subjects as seropositive or seronegative. Logistic regression was then utilized to test for differences in seroprevalence across demographic subgroups and to compare seroprevalence in cases and controls, adjusting for study design variables (gender, age, ethnic origin and study site) and education. Likewise, we used polytomous logistic regression to test for differences in seroprevalence between cases with various NHL subtypes and controls. Analyses were also conducted to examine the prevalence of high-level JCV antibody among cases and controls.

We used similar statistical methods to characterize antibody patterns and viral shedding in homosexual men. In analyzing longitudinal changes in antibody levels among treated NHL patients, we tested for changes in paired results using the Wilcoxon signed-rank test.

Results

JCV and BKV antibody responses in NHL cases and controls

Table I shows that NHL cases and controls from the NCI-SEER case-control study included in the present investigation were well matched. Cases and controls were similar in terms of gender, study center and education, although they tended to differ by ethnic origin (p = 0.08), with controls more likely than cases to be African-American (8.4% *vs.* 5.5%). On average, cases were slightly younger than controls (mean age = 56.7 *vs.* 58.1 years, p = 0.04). Among cases, the median time from NHL diagnosis to blood sampling was 153 days (interquartile range = 107–258).

TABLE II – JC AND BK VIRUS SEROPREVALENCE AMONG CONTROLS FROM THE NCI-SEER CASE-CONTROL STUDY OF NON-HODGKIN LYMPHOMA

Category	Subjects	JCV seropositive		BKV seropositive	
		n (%)[1]	*p*-value[2]	*n* (%)[1]	*p*-value[2]
Overall	622	367 (59)		401 (64)	
Age, years			0.008		0.03
20–34	39	15 (39)		26 (67)	
35–44	60	36 (60)		42 (70)	
45–54	115	65 (57)		82 (71)	
55–64	137	76 (55)		91 (66)	
65–74	271	175 (65)		160 (59)	
Gender			0.25		0.28
Male	339	207 (61)		225 (66)	
Female	283	160 (57)		176 (62)	
Ethnic origin			0.85		0.04
Caucasian	536	317 (59)		342 (64)	
African-American	52	29 (56)		41 (79)	
Other	34	21 (62)		18 (53)	
Site			0.12		0.14
Detroit	66	35 (53)		42 (64)	
Iowa	212	130 (61)		146 (69)	
Los Angeles	129	85 (66)		87 (67)	
Seattle	215	117 (54)		126 (59)	
Education, years			0.11		0.42
0–11	54	34 (63)		31 (57)	
12–15	374	228 (61)		243 (65)	
16 or more	194	105 (54)		127 (65)	

[1]Seropositivity for JC virus and BK virus was defined using a virus-like particle enzyme immunoassay (absorbance >0.12 optical density units). JCV, JC virus; BKV, BK virus.–[2]*p*-Values were calculated in univariate logistic regression models. *p*-Values for age and education were calculated for trend across categories.

Table II shows JCV and BKV seroprevalence among population-based controls from the NCI-SEER study. JCV seroprevalence increased with age ($p = 0.008$) whereas BKV seroprevalence decreased ($p = 0.03$). JCV seroprevalence did not vary by ethnic origin, but BKV seroprevalence was higher in African-Americans than other ethnic groups ($p = 0.04$, Table II). For both viruses, seroprevalence did not vary significantly by gender, study site or education.

Notably, the level of JCV antibody was significantly lower in NHL cases than controls (median OD = 0.12 *vs.* 0.21, $p < 0.0001$; Fig. 1). The difference in JCV antibody was reflected in a significantly lower JCV seroprevalence in cases than controls (49% *vs.* 59%, age-, gender-, ethnic origin-, and site-adjusted odds ratio [OR] = 0.70, 95% confidence interval [CI] = 0.56–0.87). Further adjustment for education did not affect this result (adjusted OR = 0.69, 95%CI = 0.55–0.86). This relationship seemed constant across NHL defined by histologic subtype, nodal *vs.* extranodal status, and treatment status (Table III). In contrast, the level of BKV antibody did not differ in cases and controls (median OD = 0.21 *vs.* 0.22, $p = 0.40$; Fig. 1), and cases and controls had identical BKV seroprevalence (65% *vs.* 65%; age-, gender-, ethnic origin-, and site-adjusted OR = 0.96, 95%CI =0.77–1.21).

Among cases, JCV and BKV seroprevalence did not vary with time from NHL diagnosis. JCV seroprevalence was 51% for the 248 cases sampled <120 days after diagnosis, 47% for the 273 cases sampled 120–239 days after diagnosis, and 51% for the 203 cases sampled 240+ days after diagnosis ($p = 0.50$). For BKV, the corresponding seroprevalence estimates were 66%, 66%, and 62% ($p = 0.66$).

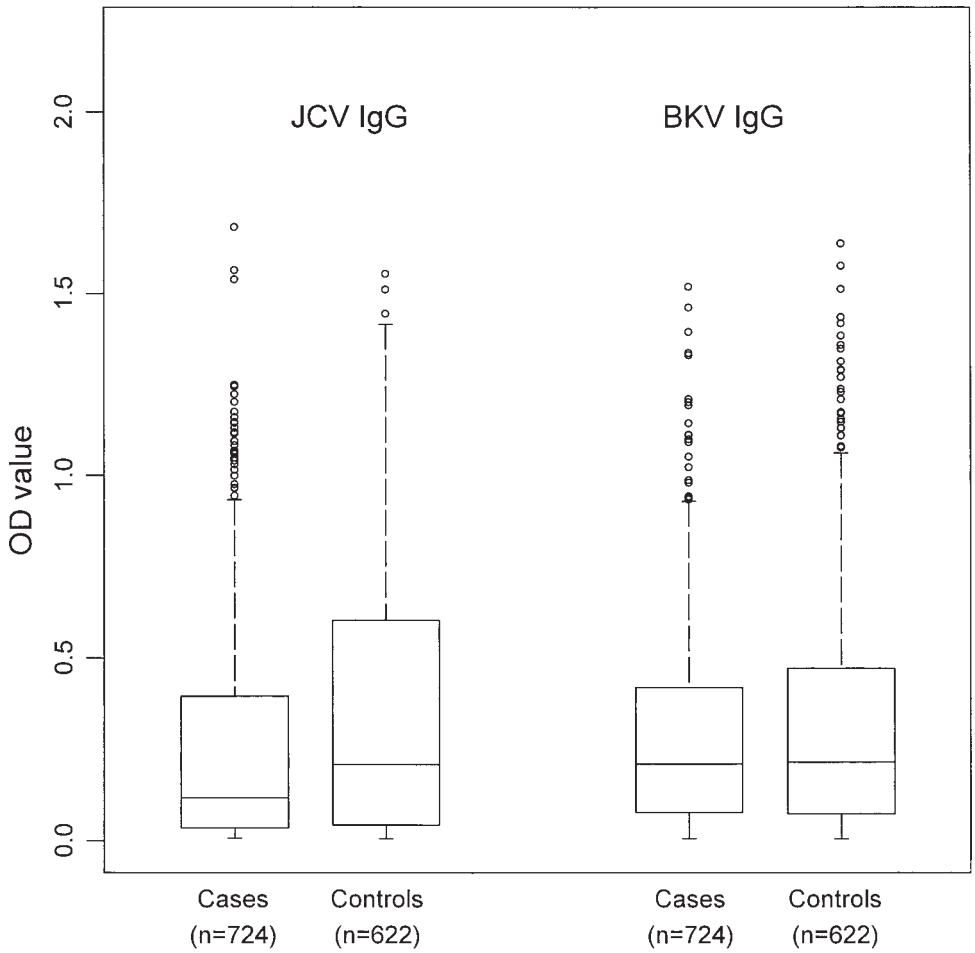

FIGURE 1 – Antibodies to JC virus and BK virus in non-Hodgkin lymphoma cases and controls. IgG antibodies were measured to viral capsids using virus-like particle enzyme immunoassays. Results are shown for 724 cases and 622 controls from the NCI-SEER case-control study of non-Hodgkin lymphoma. Results are shown as box plots: the horizontal line represents the median, and the box depicts the interquartile range. Lines extend outward from the box for 1.5 times the interquartile range, and points outside this range are shown individually.

TABLE III – ASSOCIATION BETWEEN JC VIRUS ANTIBODY LEVEL AND NON-HODGKIN LYMPHOMA CASE-CONTROL STATUS[1]

Category	Subjects	JCV antibody level OD value			JCV seropositive		High-level JCV antibody	
		Median	Interquartile range	*p*-value[2]	*n* (%)	Adjusted OR (95% CI)[3]	*n* (%)	Adjusted OR (95% CI)[3]
Controls	622	0.21	0.04–0.60		367 (59)		270 (43)	
Cases	724	0.12	0.03–0.40	<0.0001	357 (49)	0.70 (0.56–0.87)	211 (29)	0.54 (0.43–0.68)
Cases by NHL histology								
Follicular	184	0.13	0.05–0.47	0.06	96 (52)	0.85 (0.61–1.19)	58 (32)	0.64 (0.45–0.91)
Diffuse large B cell	214	0.10	0.03–0.36	<0.0001	101 (47)	0.66 (0.48–0.91)	61 (29)	0.53 (0.37–0.74)
T cell	47	0.13	0.06–0.34	0.17	24 (51)	0.77 (0.42–1.41)	13 (28)	0.52 (0.27–1.02)
Other/unknown[4]	279	0.11	0.03–0.41	0.0003	136 (49)	0.64 (0.48–0.85)	79 (28)	0.49 (0.36–0.67)
Cases by NHL site								
Nodal	481	0.10	0.03–0.35	<0.0001	229 (48)	0.66 (0.52–0.84)	134 (28)	0.51 (0.39–0.66)
Extranodal[5]	243	0.14	0.04–0.45	0.02	128 (53)	0.79 (0.58–1.06)	77 (32)	0.60 (0.44–0.82)
Cases by NHL treatment								
No prior treatment	121	0.15	0.04–0.46	0.10	65 (54)	0.79 (0.53–1.17)	40 (33)	0.60 (0.39–0.91)
Prior treatment[6]	603	0.11	0.03–0.37	<0.0001	292 (48)	0.68 (0.54–0.86)	171 (28)	0.53 (0.41–0.67)

[1]JC virus seroprevalence was defined as an absorbance >0.12 optical density units on a virus-like particle enzyme immunoassay; seroprevalence was then the proportion who are seropositive. High-level JCV antibody was defined as an absorbance >0.30 optical density units. JCV, JC virus; OR, odds ratio; CI, confidence interval; NHL, non-Hodgkin lymphoma.–[2]*p*-Values compare each NHL subgroup with the controls and were calculated using the Wilcoxon rank sum test.–[3]OR compare each NHL subgroup with the controls and were calculated using polytomous logistic regression, adjusting for age, gender, ethnic origin and study site. For high-level antibody, the OR compares subjects with high-level antibody to those without high-level antibody (both seropositive and seronegative subjects).–[4]Includes cases histologically classified as small lymphocytic ($n = 78$), lymphoplasmacytic ($n = 13$), mantle zone ($n = 30$), Burkitt ($n = 12$), marginal zone ($n = 21$), mucosa-associated lymphoid tissue ($n = 43$), or not otherwise specified ($n = 82$) subtype.–[5]Fourteen NHL cases with unknown site are included in the extranodal category.–[6]One NHL case with unknown treatment status is included in the category of those with prior treatment.

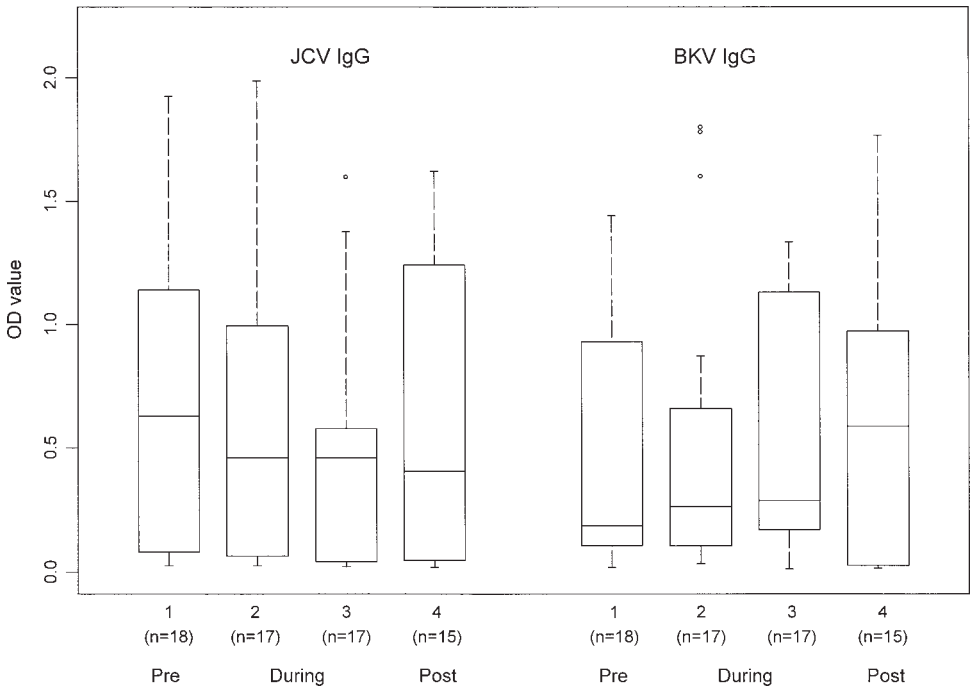

FIGURE 2 – Antibodies to JC virus and BK virus among non-Hodgkin lymphoma patients receiving chemotherapy. As in Figure 1, IgG antibodies were measured to viral capsids using virus-like particle enzyme immunoassays, and results are shown as box plots. Data are from a study of 24 non-Hodgkin lymphoma patients followed longitudinally during chemotherapy. Timepoints correspond to: 1, pre-therapy; 2 and 3, during therapy; and 4, post-therapy.

Figure 1 suggests that the lower level of JCV antibody among cases relative to controls was due, in large part, to a difference in the proportion of subjects with especially high levels of JCV antibody. A cutpoint of 0.30 OD units to define high-level JCV antibody was suggested by our analyses of polyomavirus shedding. Using this cutpoint, the prevalence of high-level JCV antibody was inversely associated with NHL overall (adjusted OR = 0.54, 95%CI = 0.43–0.68, for high-level antibody *vs.* lower-level antibody or seronegative) and various NHL subtypes (Table III). The prevalence of high-level antibody was 30% for NHL cases sampled <120 days after diagnosis, 27% for cases sampled 120–239 days after diagnosis and 30% for cases sampled 240+ days after diagnosis ($p = 0.74$).

Serial JCV and BKV antibody measurements for 24 other NHL patients undergoing chemotherapy are displayed in Figure 2. There was a marginally significant decline in JCV antibody levels from the first timepoint, before therapy, to the second timepoint, during therapy (median decline = 0.09 OD units, $p = 0.05$ based on $n = 13$ paired observations). JCV antibody levels did not change subsequently (second *vs.* third timepoint, also during therapy, $n = 13$ pairs, $p = 0.24$; third *vs.* fourth timepoint, after the end of therapy, $n = 11$ pairs, $p = 0.83$). BKV antibody levels did not change over time (first *vs.* second timepoint, $p = 0.74$; second *vs.* third timepoint, $p = 0.38$; third *vs.* fourth timepoint, $p = 0.64$).

Relationship between JCV and BKV shedding and antibody levels

To clarify the utility of JCV antibody levels as a marker for active viral replication, we studied 126 homosexual men. Forty-six (37%) of these men were shedding JCV in their urine, indicating active viral replication. JCV shedding was unrelated to HIV infection status ($p = 0.97$), or to CD4 count among those with HIV

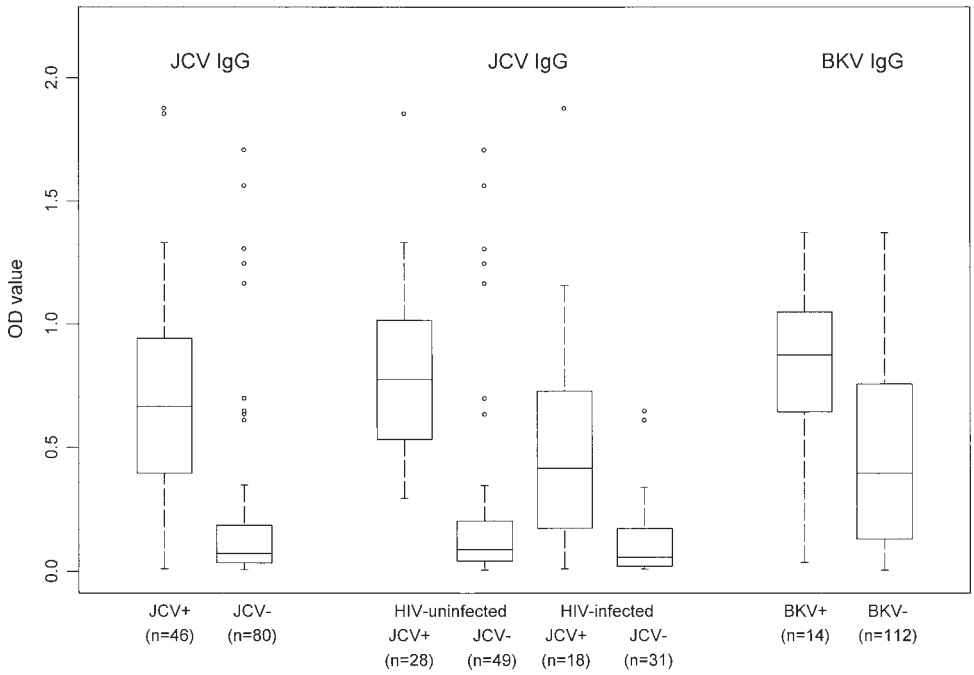

FIGURE 3 – Antibodies to JC virus and BK virus among homosexual men. As in Figure 1, IgG antibodies were measured to viral capsids using virus-like particle enzyme immunoassays, and results are shown as box plots. Data are from a study of 126 homosexual men, of whom 49 were infected with human immunodeficiency virus. JCV+/− and BKV+/− refer to the shedding/non-shedding of these viruses in urine specimens obtained concurrently with serum samples measured for antibodies.

infection (p-trend = 0.62). JCV antibody levels were higher in shedders than non-shedders (median OD = 0.67 *vs.* 0.07, $p < 0.0001$; Fig. 3). JCV antibody levels were lower for HIV-infected shedders of JCV than for HIV-uninfected shedders ($p = 0.007$, Fig. 3). Nonetheless, the association between JCV antibody and shedding was apparent in both groups (Fig. 3). JCV seroprevalence was higher in shedders than non-shedders (93% *vs.* 40%, $p < 0.0001$). Using a cutoff of 0.30 OD units, 85% of shedders but only 15% of non-shedders had high-level JCV antibody.

Only 14 (11%) homosexual men were shedding BKV. BKV shedding was more common in HIV-infected men than HIV-uninfected men (20% *vs.* 5%, $p = 0.01$). As was observed for JCV, BKV antibody levels were higher in BKV shedders than non-shedders (median OD = 0.87 *vs.* 0.40, $p = 0.003$; Fig. 3). This relationship held for HIV-infected and HIV-uninfected men (not shown). BKV seroprevalence was higher in BKV shedders than non-shedders, although this difference was not significant (93% *vs.* 76%, $p = 0.18$).

Discussion

The major finding of our study was the presence of lower levels of IgG antibody against JCV capsid among NHL cases compared to controls (Fig. 1). The overall decreased levels in NHL cases manifested as both a reduced prevalence of individuals considered JCV seropositive (*i.e.*, seroprevalence) and a reduced prevalence of individuals with high-level JCV antibody. Furthermore, the pattern was consistent across NHL subtypes (Table III). Our finding was unexpected and is somewhat difficult to explain, as we could not identify a mechanism by which JCV could protect against NHL. Chance is a theoretical explanation, but the decrease in JCV antibody was highly statistically significant. We consider 4 possible explanations for the decreased JCV antibody levels seen in NHL cases: (*i*) extraneous differences in NHL cases and controls that could lead to differences in JCV infection status (confounding); (*ii*) broadly disordered antibody production in persons with NHL (disease effect); (*iii*) an effect of NHL treatment on antibody levels (treatment effect); and (*iv*) an etiological role for JCV in NHL.

Confounding would explain decreased JCV seroprevalence in NHL cases if determinants of JCV infection materially differed in cases and controls. It is difficult to fully evaluate this possibility, because the epidemiology of JCV and BKV is not well understood, and routes of transmission are unknown. Consistent with other published data,[1–3] we found that JCV seroprevalence increased with age during adulthood, whereas BKV seroprevalence was already somewhat high among young adults. Our results suggest that BKV is more readily acquired in childhood than is JCV. Indeed, as others have also reported,[1,3] we found that BKV seroprevalence was lower in older individuals, possibly reflecting a decline in antibody levels with aging. We did not see differences in JCV seroprevalence by ethnic origin or education, suggesting that risk of infection does not depend on socioeconomic status. Importantly, the inverse association between JCV serostatus and NHL was present after adjustment for demographic factors, indicating that factors related to risk for acquiring JCV infection are unlikely to be important confounders.

We cannot rule out that the reduced JCV antibody levels in NHL cases may simply reflect, at least in part, dysfunctional antibody production arising from NHL itself. The possiblity of a disease effect is raised by our results for HIV-infected homosexual men, who showed lower JCV antibody than HIV-uninfected men (Fig. 2). There are few published data regarding antibody production in persons with NHL,[17,18] which suggest a modest reduction in overall IgG levels related to NHL, perhaps limited to certain NHL subtypes or to especially advanced disease. Nonetheless, the best argument against a major disease effect in the present instance is provided by our data on BKV, a virus closely related to JCV: the similarity in BKV antibody levels between cases and controls would indicate that persons with NHL can make appropriate levels of IgG antibody, at least in response to chronic infections with polyomaviruses.

To evaluate the effect of chemotherapy on antibodies, we longitudinally studied NHL patients receiving EPOCH chemotherapy. BKV antibody levels did not decline during therapy. We did find a small decrease in JCV antibody level between the pre-treatment and first on-treatment serum samples, but no further decline was seen with additional doses of chemotherapy (Fig. 3). In our case-control study, JCV and BKV seroprevalence, and the prevalence of high-level JCV antibody, did not depend on the interval since NHL diagnosis. Similarly, the inverse associations of JCV seropositivity and high-level JCV antibody with NHL were seen even for untreated

cases. Our results suggest that an effect of NHL treatment does not entirely explain our main finding. Ideally, additional studies will further clarify the degree to which antibody production is abnormal in NHL patients, either due to the disease or its treatment.

Several lines of evidence suggest that JCV could play a role in the development of NHL and bear on an etiologic interpretation of our data. First, JCV can infect B lymphocytes.[14,19] Second, JCV infection within cells can induce genomic instability,[8] and intracellular expression of the viral T antigen protein, which inactivates the p53 tumor suppressor protein, can prevent damaged cells from undergoing apoptosis.[20] Third, Neel *et al*.[9] and Lazutka *et al*.[21] showed that individuals whose circulating lymphocytes manifest major chromosomal abnormalities have elevated antibody titers against JCV; these observations might suggest that JCV antibody levels would be elevated later among individuals who develop NHL, which would be opposite of our findings. Finally, molecular evidence that JCV could be important in some NHL was provided by Del Valle *et al*.,[22] who reported recently the detection of JCV in 22 (81%) of 27 central nervous system (CNS) NHL. Two subsequent studies, however, could not confirm those findings.[23,24] Several other research groups have searched for polyomavirus DNA in tumor tissue from systemic (extra-CNS) NHL. Although some detected SV40 DNA, a controversial finding (*c.f.* our recent analysis of SV40 antibody responses in the NCI-SEER case-control study),[13] none found JCV DNA.

Khalili *et al*.[25] have proposed a ''hit-and-run'' mechanism whereby JCV could cause colon cancer, and it is conceivable that a similar model could apply for NHL. Under such a model, JCV infection of lymphocytes could lead to persistent chromosomal damage. Damaged lymphocytes might acquire additional genetic ''hits'' over time, even after loss of JCV from the cell,[8] and the accumulating damage might eventually lead to neoplasia. Although controversial and unproven, similar hit-and-run models have been proposed for other hypothesized virus-cancer associations.[26,27] Along these lines, we showed that antibodies against JCV capsid, especially at higher levels, are a marker for JCV replication (Fig. 3), so the lower levels of JCV capsid antibody in NHL cases might point to a relative absence of JCV replication among NHL cases. Still, it is unclear how to formulate a model whereby JCV replication is actually reduced after hit-and-run damage to lymphocytes.

Other scenarios are possible. The low level of JCV antibody among NHL cases could reflect an innate defect in immunity, perhaps specific to JCV. This defect could prevent some persons from effectively controlling JCV infection, manifesting as a relative inability to produce JCV antibody and predisposing to lymphocyte infection or proliferation. Alternatively, the defect in immunity could be acquired, perhaps as a result of ''viral interference,'' whereby simultaneous infection with JCV and another virus would prevent an adequate immune response against JCV.

The lack of firm data to choose among these possible explanations highlights limitations of our study and identifies at least 2 specific opportunities for future research into the role of JCV in NHL development. First, the only biomarker of JCV and BKV infection that we measured was IgG antibody against the viral capsids. It would also be useful to examine cellular immune responses against these viruses, and to directly examine whether JCV replication and shedding are decreased in persons with NHL. Second, we only had samples from individuals who already had NHL. To examine whether JCV replication is an initiating event that might long precede NHL, one could examine JCV antibody levels in serum samples obtained before NHL diagnosis. It would be informative to replicate the findings of Neel *et al*.[9] and Lazutka *et al*.[21] using newer antibody assays for JCV infection and chromosomal damage.

In conclusion, our study demonstrated a specific deficit in IgG antibody to JCV capsid in persons with NHL. Although our findings could be explained by effects on antibody levels that are intrinsic to NHL or that result from its treatment, these explanations may be incomplete. Our results provide an opportunity to consider the intriguing possibility that JCV could be involved in the etiology of NHL. More laboratory and epidemiological research is needed to test this hypothesis.

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